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Dear Sir or Madam:

As required by 40 CFR 716, as amended, we herewith submit a copy of the following recently completed health and safety study.

Determination of the LC50 (14 of TDI, TDA, MDI & MDA to the
earthworm *Eisenia fetida* accordi. OECD Guideline no. 207 (TNO).
Project E-CE-96.

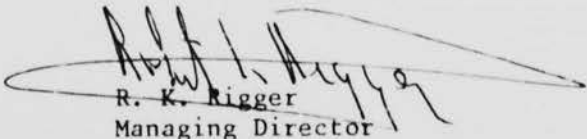
<u>Chemical Name</u>	<u>CAS Number</u>
Toluenediisocyanate	26471-62-5
Toluenediamine	25376-45-8
Polymeric diphenyl methane diisocyanate	9016-87-9
(contains 4,4'-diphenyl methane diisocyanate)	101-68-8
4,4'-diaminodiphenylmethane	101-77-9

The International Isocyanate Institute (III) project identification number, **E-CE-96**, has been marked as part of the title of this report. Please refer to this III identification number in any communication regarding this study. **The enclosed report does not contain any Confidential Business Information.**

This study is sponsored by the International Isocyanate Institute on behalf of the following:

The Dow Chemical Company
Miles, Inc.
BASF Corporation
ICI Americas, Inc.
Olin Corporation

Very truly yours,


R. K. Rigger
Managing Director

RKR/sha

44 pages

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REPORT

Title

DETERMINATION OF THE LC50 (14 DAYS) OF TDI, TDA,
MDI AND MDA TO THE EARTHWORM EISENIA FETIDA
ACCORDING TO OECD GUIDELINE NG.207

Author(s)

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TNO Institute of Environmental Sciences,
Delft, The Netherlands.

Observations

See III Report 11024 for other terrestrial studies
(Avena Sativa and Lactuca Sativa)

See also TNO notes on Report III.11028

III Project

E-CE-96

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11025

Date of III issue

93.01.25



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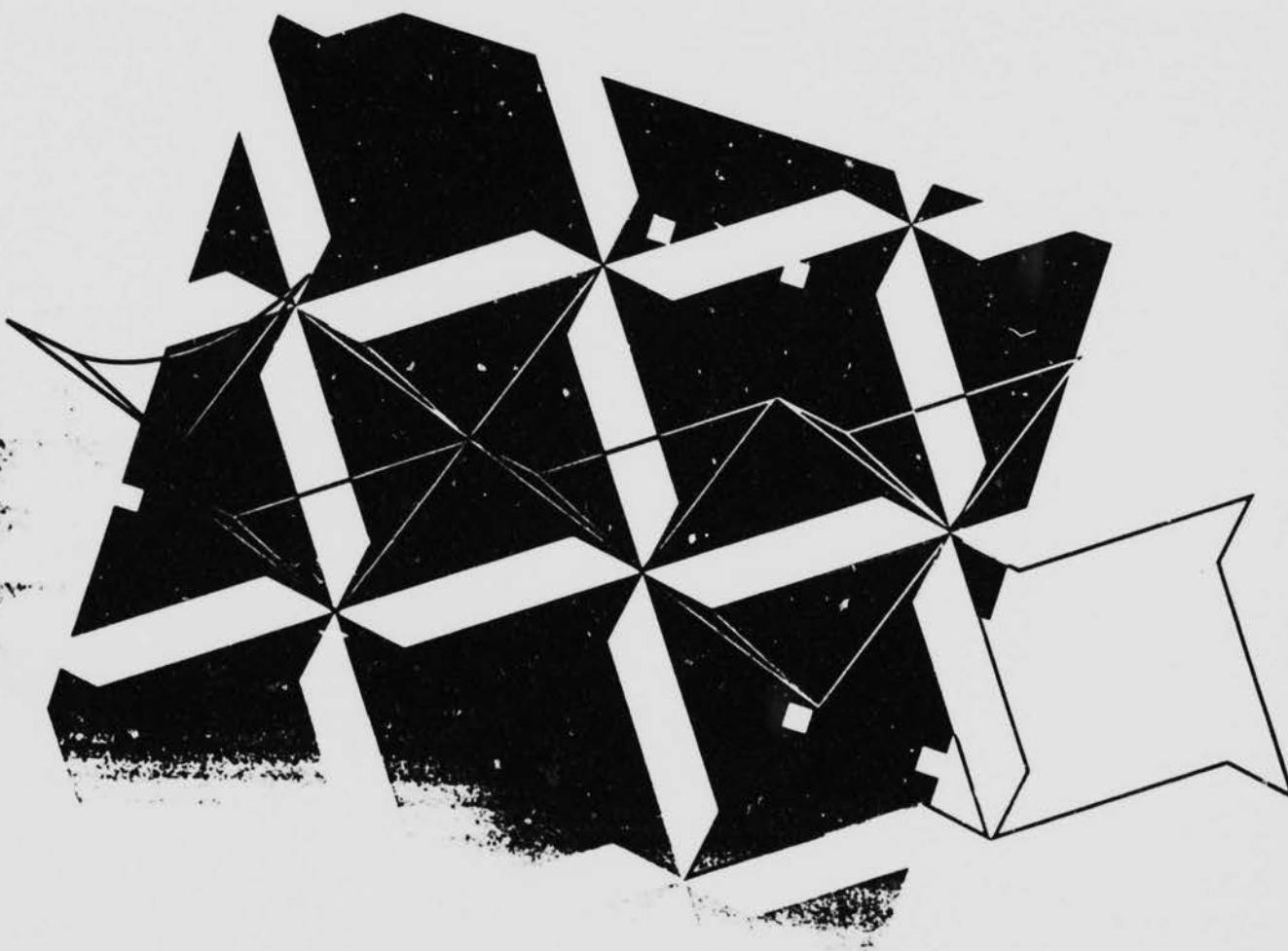
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Summary

" SEE APPENDED (pp7-8) "

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TNO-report IMW - R 92/188

Determination of the LC50 (14 days) of TDI, TDA,
MDI and MDA to the earthworm *Eisenia fetida*
according to OECD Guideline no. 207 (TNO)
Project E-CE-96

Author : Dr N. van der Hoeven
P. Roza
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GLP COMPLIANCE STATEMENT

'I, the undersigned, hereby declare that the work to which this report refers was performed under my supervision according to the procedure herein described. To the best of my knowledge this report provides an accurate record of the results obtained. The study was carried out in compliance with the OECD code of Good Laboratory Practice. Characterization and verification of the test substance identity and properties is, however, the responsibility of the sponsor.'



Dr N. van der Hoeven
Study Director

Date: 1 December, 1992

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Date: 1992-12-01



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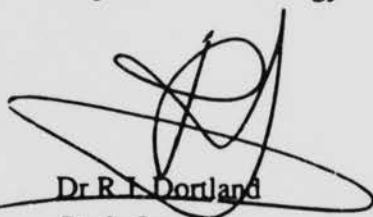
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Report no.: R92/188
Study no. : IMW-91-0032-01
IMW-91-0033-01
IMW-91-0034-01
IMW-91-0036-01

QUALITY ASSURANCE STATEMENT

STUDY TITLE: Determination of the LC50 (14 days) of TDI, TDA, MDI and MDA to the earthworm Eisenia fetida according to OECD Guideline no.207 (TNO). Project E-CE-96

REPORT DATE: November 5, 1992

The following inspections relevant to this study have been carried out by the Quality Assurance Unit of the TNO Institute of Environmental Sciences (IMW), P. O. Box 6011, 2600 JA Delft, the Netherlands.

Type of inspection	Date and number of inspections	Date of report to Study Director
protocol:	April 19, 1991 (1)	April 19, 1991
experimental phase:	February 26, 1992 (1) February 28, 1992 (1) March 13, 1992 (1) April 14, 1992 (1)	February 26, 1992 February 28, 1992 March 13, 1992 April 14, 1992
study documentation:	February 12, 1992 (1) March 4, 1992 (1)	February 13, 1992 March 4, 1992
report audit:	September 2, 1992 (1)	September 2, 1992

Any serious deviations were reported to management at the same time as the report to the study director; any other, less serious deviations were reported to management upon receipt of the reply from the Study Director.

I, the undersigned, hereby declare that to the best of my knowledge this report provides an accurate record of the results obtained in this study.



M. Ph. Van den Berg
Quality Assurance Officer

Date: December 9, 1992

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SUMMARY AND CONCLUSIONS

The acute toxicity of the substances toluene diisocyanate 80/20 (TDI), toluene diamine 80/20 (TDA), diphenyl-methane-diisocyanate (MDI) and 4,4'-diaminodiphenylmethane, laboratory product (MDA) to the worm *Eisenia fetida* were tested in accordance with the OECD Guideline 207 (ref. 1) and the Draft EC Guideline (ref. 2) and the OECD principles of Good Laboratory Practice (ref. 3).

The four test substances were tested separately.

Worms were exposed to the test substances mixed with artificial soil for a period of 14 days, after which survival was determined and the condition of the worms as to mobility and appearance was visually assessed. At the start of the experiment and after 14 days the surviving worms were individually weighed.

The concentrations of the test substances are expressed in mg per kg of the dry artificial soil. These concentrations refer to the test substance as supplied by the sponsor.

Range-finding tests were performed with the four test substances in concentrations of 0, 10, 100 and 1000 mg.kg⁻¹. Based on the results of these range-finding tests the concentrations of the final tests were chosen. In the final tests, the dosed concentrations were:

TDI : 0 and 1000 mg.kg⁻¹
TDA : 0, 46.4, 100, 215, 464 and 1000 mg.kg⁻¹
MDI : 0 and 1000 mg.kg⁻¹
MDA : 0, 18, 32, 56, 100, 320 and 560 mg.kg⁻¹

The following effect concentrations were observed in the four tests:

	TDI	TDA	MDI	MDA
14 day LC50	: >1000	>1000	>1000	444
14 day NOLC	: ≥1000	464	≥1000	180
14 day NOEC (weight increase)	: ≥1000	215	≥1000	32
14 day NOEC (behaviour and appearance)	: ≥1000	215	≥1000	56

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No effects on mortality, weight increase, behaviour or appearance were observed for the two diisocyanates (TDI and MDI) after 14 days exposure to the highest test concentration, i.e. 1000 mg per kg of dry soil.

The two diamines (TDA and MDA) appeared to be more toxic than the corresponding diisocyanates, while MDA was more toxic than TDA.

The environmental conditions during the experiments were as follows:

Temperature: $20 \pm 2^\circ\text{C}$

	TDI	TDA	MDI	MDA
Moisture content (%) at start :	54	52	53	55
Moisture content (%) at end :	53	48	49	51
pH at start :	6.2	6.4	6.3	6.2
pH at end :	6.7	6.7	6.4	6.6

(moisture content is based on dry constituents)

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1. INTRODUCTION

The acute toxicity of the substances toluene diisocyanate 80/20 (TDI), toluene diamine 80/20 (TDA), diphenyl-methane-diisocyanate (MDI) and 4,4'-diaminodiphenylmethane, laboratory product (MDA) to the worm species *Eisenia fetida* were determined at the request of the sponsor. The tests were carried out in conformity with OECD Guideline 207 (ref. 1) and the Draft EC Guideline (ref. 2) and the OECD principles of Good Laboratory Practice (ref. 3). The test substances were supplied by the sponsor.

The four test substances were tested separately.

For each test substance, the objectives of the studies were to determine, in case effects could be observed at concentrations at or below 1000 mg.kg⁻¹ of dry soil (the highest concentration normally tested according to the OECD Guideline no. 207 (ref. 1) and the Draft EC Guideline (ref. 2) in acute toxicity assays with the worm species (*Eisenia fetida*):

- the 14 days LC50 of the test substance, i.e. the concentration which kills 50% of the exposed worms in 14 days under the experimental conditions defined in section 2.4.
- the minimum concentration tested producing total mortality and the maximum concentration tested producing no mortality and preferably also the maximum concentration tested producing no visible abnormalities.

Otherwise, the objective of the studies was to determine in a limit test whether no effects could be found at a concentration of 1000 mg.kg⁻¹ of dry soil.

The effects of concentrations higher than 1000 mg of test substance per kg of dry soil were not investigated.

Relevant dates for the tests were:

TDI : Protocol (TNO study no.: GLP 91/063) signed by the Study Director on:
April 19, 1991

Amendment No. 1 to this protocol signed by the Study Director on: October 4,
1991

Period of range finding test : January 31, 1992 to February 14, 1992

Period of Final test : February 28, 1992 to March 13, 1992



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TDA : Protocol (TNO study no.: GLP 91/064) signed by Study Director on:

April 19, 1991

Amendment No. 1 to this protocol signed by the Study Director on: October 4, 1991

Period of range finding test : January 29, 1992 to February 12, 1992

Period of Final test : March 31, 1992 to April 14, 1992

MDI : Protocol (TNO study no.: GLP 91/065) signed by the Study Director on:

April 19, 1991

Amendment No. 1 to this protocol signed by the Study Director on: October 4, 1991

Period of range finding test : March, 14, 1992 to March 27, 1992

Period of Final test : April 23, 1992 to May 7, 1992

MDA : Protocol (TNO study no.: GLP 91/067) signed by the Study Director on:

April 19, 1991

Amendment No. 1 to this protocol signed by the Study Director on: October 4, 1991

Period of range finding test : January 31, 1992 to February 14, 1992

Period of Final test : February 28, 1992 to March 13, 1992



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2. MATERIALS AND METHOD

2.1 Test substance

The test substances were toluene diisocyanate 80/20 (TDI), toluene diamine 80/20 (TDA), diphenyl-methane-diisocyanate (MDI) and 4,4'-diaminodiphenylmethane, laboratory product (MDA). The test substance will be indicated in this report by the abbreviations, TDI, TDA, MDI and MDA respectively.

For these tests the following batches of test substance were used:

- TDI :** The batch of test substance was received on July 2, 1991 in a 1 litre aluminium screw-capped bottle. This bottle was labelled: 'Desmodur T80 Giftig 2,4/2,6-diisocyanat-toluol., Datum: 20.6.1991, Partie: 808, Tank: 6, Referenz: IMW 91/746. The test substance came in the form of a colourless to yellowish liquid. The test substance was stored at room temperature, protected from light in a closed cupboard. According to the sponsor, TDI contained 80% of the 2,4 isomer and 20% of the 2,6 isomer of toluene diisocyanate and its purity was more than 99.9%. TDI was stated to react with water and to be soluble in acetone.
- TDA :** The batch of test substance was received on July 2, 1991 in a 1 litre aluminium screw-capped bottle. This bottle was labelled: 'M-TDA, Giftig 2,4 u 2,6-diaminotoluol., 4.6.91, PT.12, Referenz: IMW 91/746. The test substance came in the form of a brown solid. The test substance was stored at room temperature, protected from light in a closed cupboard. According to the sponsor the batch contained more than 99% active ingredient, i.e. toluene diamine. The water solubility of TDA was stated to be about 100 g.l⁻¹.
- MDI :** The batch of test substance was received on February 24, 1992 in a 1 litre aluminium screw-capped bottle. This bottle was labelled: '4,4' diphenylmethan-diisocyanat, isomere/homologe, harmful, Bayer AG'. The test substance came in the form of a dark-brown liquid. The test substance was stored at room temperature, protected from light in a closed cupboard. According to the sponsor the active ingredients of MDA were diphenyl-methane-diisocyanate (isomers and homologous) and consisted of 40-50% of the 4,4'-isomer, 2-4% of the 2,4'-isomer and 40-60% of 3-ring isomers. MDI contained traces of phenylisocyanate and



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monochlorobenzene as impurities. MDI was stated to react with water, forming urea and CO_2 and to be soluble in acetone.

MDA : The batch of test substance was received on January 20, 1992 in a 1 litre square glass bottle with a blue screw-cap. This bottle was labelled: 'Referenz IMW 91/746, 4,4'-diamino-diphenylmethane, BMC 200/10: MDA 100 dest'. The test substance came in the form of a colourless to light yellow solid lump. The test substance was stored at room temperature, protected from light in a closed cupboard. According to the sponsor its purity was more than 99.5% of the active ingredient, 4,4'-diaminodiphenylmethane (laboratory product). MDA contained traces of 2,4'-diaminodiphenylmethane and higher molecular weight oligomers as impurities. MDA is stated to be practically insoluble in water and to be soluble in acetone.

The composition and properties of the four test substances as specified by the sponsor are recorded in Annex A.

2.2 Test organism

The test organism was the worm species *Eisenia fetida*, grown in the laboratory in a horse-manure garden soil (1:1) mixture at about 23°C. Their weight (per worm), and its standard deviation, were measured at the beginning and end of the test (see Table B1). The worms had been grown from stock originally supplied by NLAC-proefdierbedrijf, Millseweg 1, Beers (N-B), the Netherlands.

2.3 Artificial soil

The artificial soil consisted of finely ground (no visible plant remains) sphagnum peat, kaolin clay and fine industrial sand in a ratio of 1:2:7 (based on dry weight) (for details see annex C). Some calcium carbonate is added to the soil to adjust the final pH of the mixture to 6.0 ± 0.5 .

For test substances which were sufficiently soluble to be dosed directly in water to the relatively dry artificial soil, all components were mixed in a small electric cement mixer before addition of the test substance. However, only TPA could be dosed in this manner.

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Test substances which could be dissolved in acetone, were prepared by another method. A kaolin clay-sphagnum peat mixture was prepared and thoroughly mixed with a hand mixer. An appropriate amount of industrial sand was coated with the test substance and thereafter mixed through sufficient kaolin clay-sphagnum peat mixture. Soils with TDI, MDI and MDA were prepared in this way. After addition of the test substance, water was added to yield a final water content (based on dry constituents) of about 55%.

2.4 Test method

The test was conducted in accordance with the OECD Guideline no. 207 (ref. 1) and the Draft EC Guideline (ref. 2). Range-finding tests were performed with the four test substances to determine the test concentrations in the final test. For TDI and MDI no effects were observed in these range-finding tests at a concentration of 1000 mg.kg⁻¹ of dry soil. Therefore, these two test substances were tested in a limit test.

The preparation of the test medium is described for each test substance separately.

The moisture content recorded is always based on dry constituents.

2.4.1 Preparation of test medium with TDI

TDI was tested in a limit test, i.e. only controls and a test substance concentration of 1000 mg per kg dry soil were tested. A quantity of 2506 mg of TDI was accurately weighed and dissolved in 500 ml acetone. From this solution 100 ml was added to 350 g dry fine industrial sand in the test containers. After mixing, this was left to dry for two days in a fume-cupboard, after which time the acetone had evaporated.

A kaolin clay-sphagnum peat mixture with a dry weight ratio of 2:1 was prepared by mixing 4.70 kg dry kaolin clay with 4.83 kg wet sphagnum peat (consisting of 2.3 kg dry sphagnum peat and 2.53 kg water, moisture content 109.8%) and 93.02 g CaCO₃. A quantity of 207.4 g of this kaolin clay - sphagnum peat mixture (dry weight 152 g) was added to the test container to reach a concentration of 1000 mg TDI per kg of dry soil. Controls were prepared in a similar manner by adding 100 ml of pure acetone to 350 g of sand.

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The test containers were shaken thoroughly to mix the sand with the sphagnum peat - clay mixture. A quantity of 219.8 ml demineralized water was then added to the test container. The water was left to soak into the artificial soil, which was not stirred or mixed after this addition.

Five containers with 1000 mg TDI per kg of dry soil and five containing control soil were separately prepared in this way.

2.4.2 Preparation of test medium with TDA

A quantity of 4979 mg of TDA was accurately weighed and dissolved in 1 l demineralized water. Of this stock solution, 23.2, 50, 107.5 and 232 ml were diluted with demineralized water to 500 ml in order to obtain solutions of 232, 500, 1075 and 2320 mg per litre. From these solutions, samples of 100 ml were diluted to 107 ml with demineralized water. These samples of 107 ml were added to pots containing 667.5 g of artificial soil with a moisture content of 33.5% (consisting of 500 g dry soil and 133.5 g water) to reach concentrations of 46.4, 100, 215, 464 and 1000 mg of TDA per kg dry soil. Controls were prepared in a similar manner by adding 107 ml of demineralized water to pots containing the same amount of soil. Five containers with 46.4, 100, 215, 464 and 1000 mg TDA per kg of dry soil and five containing control soil were separately prepared in this way.

2.4.3 Preparation of test medium with MDI

MDI was tested in a limit test, i.e. only controls and a test substance concentration of 1000 mg per kg dry soil were tested. A quantity of 2550 mg of MDI was accurately weighed and dissolved in 510 ml acetone. From this solution 100 ml was added to 350 g dry fine industrial sand in the test container. After mixing, this was left to dry for two days in a fume-cupboard, after which time the acetone had evaporated.

A kaolin clay - sphagnum peat mixture with a dry weight ratio of 2:1 was prepared by mixing 1000 g dry kaolin clay with 1025.6 g wet sphagnum peat (consisting of 500 g dry sphagnum peat and 525.6 g water, moisture content 105.1%) and 15 g CaCO_3 . 202.55 g of this kaolin clay - sphagnum peat mixture (dry weight 150 g) was added to the test container to reach a test concentration of 1000 mg MDI per kg of dry soil. Controls were prepared in a similar manner by adding 100 ml of pure acetone to 350 g sand.

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The test containers were shaken thoroughly to mix the sand with the sphagnum peat - clay mixture. A quantity of 222 ml demineralized water was then added to the test container. The water was left to soak into the artificial soil, which was not stirred or mixed after this addition.

Five containers with 1000 mg MDI per kg of dry soil and five containing control soil were separately prepared in this way.

2.4.4 Preparation of test medium with MDA

A quantity of 5130 mg of MDA was accurately weighed and dissolved in 513 ml acetone. Of this stock solution 4.5, 8.0, 14.0, 25.0, 45.0, 80.0 and 140 ml were taken and diluted with acetone to 500 ml in order to obtain solutions of 90, 160, 280, 500, 900, 1600 and 2800 mg per litre of acetone. From these solutions 100 ml was added to 350 g dry fine industrial sand in the test container. After mixing, this was left to dry for two days in a fume-cupboard, after which time the acetone had evaporated.

A kaolin clay-sphagnum peat mixture with a dry weight ratio of 2:1 was prepared by mixing 4.70 kg dry kaolin clay with 4.83 kg wet sphagnum peat (consisting of 2.3 kg dry sphagnum peat and 2.53 kg water, moisture content 109.8%) and 93.02 g CaCO_3 . 207.4 g of this kaolin clay - sphagnum peat mixture (dry weight 152 g) was added to the test container to reach test concentrations of 18, 32, 56, 100, 180, 320 and 560 mg MDA per kg of dry soil. Controls were prepared in a similar manner by adding 100 ml of pure acetone to 350 g sand.

The test containers were shaken thoroughly to mix the sand with the sphagnum peat - clay mixture. A quantity of 219.8 ml demineralized water was then added to the test container. The water was left to soak into the artificial soil, which was not stirred or mixed after this addition.

Five containers with 18, 32, 56, 100, 180, 320 and 560 mg MDA per kg of dry soil and five containing control soil were separately prepared in this way.

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2.4.5 Test conditions and measurements

A series of five containers was prepared per test substance concentration and per control. Ten worms were added to each of four of these series. The fifth container of each series was used for pH and moisture content measurement.

The test containers were 1.5 l all-glass preservers. During the tests, the lid was down but not closed.

The tests were carried out at $20 \pm 2^\circ\text{C}$ and under continuous low intensity illumination (400 to 500 lux).

At the start of each test, the pH and moisture content of the control soils were determined (to measure the pH, 50 g of soil was added to 100 ml of 0.1 M KCl, and the pH of the supernatant determined after one hour). The pH at the start of the tests with TDI, TDA, MDI and MDA were found to be 6.2, 6.4, 6.3 and 6.2 respectively.

The moisture contents at the start of the tests with TDI, TDA, MDI and MDA were found to be 54, 52, 53 and 55% (based on dry constituents) respectively.

The tests lasted two weeks, the mortality of the worms being determined at the end of 14 days exposure. On the 7th day, the burrowing behaviour of the worms was assessed. It was recorded when dead worms could be seen through the glass pots. The containers were not, however, opened or emptied. On the 14th day, the weight of the individual worms was determined.

At the end of each test, the pH and moisture content of the control soils were determined.

At the end of the test with TDI, TDA, MDI and MDA the pH's were found to be 6.7, 6.7, 6.4 and 6.6 and the moisture contents 53, 48, 49 and 51% (based on dry constituents) respectively.

2.5 Treatments of the results

2.5.1 LC50 values

The effect of a test substance on the mortality of animals is expressed by a quantity denoted as the LC50 (= Lethal Concentration, 50%), i.e. the exposure concentration of the



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substance which would prove lethal to 50% of an infinite population of the exposed animals. The LC50 is qualified according to the duration of exposure.

The tests with TDI and MDI, however, were not designed to calculate an LC50 value. (Because no mortality was expected at 1000 mg.kg⁻¹, only that one concentration was tested). For TDA and MDA, the LC50 values and their confidence intervals were calculated by means of a parametric model developed by Kooijman (ref. 3). A summary of this method is given in Annex D. The mortality data per test concentration as recorded in Annex B, Table B1.2 and B1.4 were used for these calculations.

2.5.2 NOEC values

The 'no observed effect concentrations' (NOEC values) are the highest concentrations tested showing no effects (defined below) throughout the exposure time. The NOEC values were estimated by comparing effects on mortality, weight, behaviour and appearance (the latter two visually assessed) of the exposed animals with those of the control animals (blanks). The NOEC value for mortality is also called the NOLC (no observed lethal effect concentration).

To determine the NOEC for mortality, the survival dates of each concentration were compared pair-wise with those in the control using a binomial test for comparison of proportions in two independent samples (2x2 contingency table). A significance level of 5% was used.

To determine the NOEC for weight increase, a multiple comparison was made between the average weight increase of the worms per container at each container and the average weight increase in the controls using a two-tailed Dunnett test. A significance level of 5% was used.

The NOEC was determined as follows:

- At the NOEC no significant differences with the controls were observed.
- At the first higher test concentration (LOEC; lowest observed effect concentration) a significant difference with the controls was observed.
- At all higher concentrations tested, the differences with the controls were either also significant or larger than those at the LOEC.

The NOEC for behaviour and appearance was not determined statistically.



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2.5.3 LC100 values

The LC100 (=Lethal Concentration to 100% of the test animals) is the lowest test concentration at which all animals died (provided that all animals died at all higher concentrations tested).



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3. RESULTS

The results of the tests, expressed as LC50, LC100, NOLC and NOEC values are presented in table 1. The number of living worms at the beginning and at the end of the tests are listed in Annex B, Tables B1.1 to B1.4, together with the average live weight at these observation times and the visually estimated condition (appearance and behaviour). The observed condition of the soil after 7 days is also recorded in these tables. The individual wet weight of the worms at the beginning and at the end of the tests are listed in Annex B, Tables B2.1 to B2.4

Table 1 Results of the tests with TDI, TDA, MDI and MDA and *Eisenia fetida*

Parameter	Effect	Nominal concentration (mg.kg ⁻¹ dry soil)			
		TDI	TDA	MDI	MDA
14 days LC50	mortality	-- 5)	>1000 6)	-- 5)	444 4)
14 days LC100	mortality	-- 7)	-- 8)	-- 7)	>560 1)
14 days NOLC	mortality	≥1000 1)	464 2)	≥1000 1)	180 3)
14 days NOEC	weight increase/decrease	≥1000 1)	215	≥1000 1)	32
14 days NOEC	behaviour and appearance	≥1000 1)	215	≥1000 1)	56

1) Highest concentration tested.

2) Four worms died, but mortality was not significantly higher than in controls.

3) One worm died, but mortality was not significantly higher than in controls.

4) 95% confidence interval = 390-500 mg.kg⁻¹ dry soil.

5) LC50 could not be determined, since no effects on mortality were observed even at the highest concentration tested.

6) Highest concentration tested; the model estimate of LC50 was outside the concentration range tested (1050 mg.kg⁻¹).

7) Even at the highest concentration tested (1000 mg.kg⁻¹) no effects on mortality were observed (100% survival).

8) Even at the highest concentration tested (1000 mg.kg⁻¹), mortality was less than 50%.

No effects on mortality, weight increase, behaviour or appearance were observed after 14 days exposure to the highest test concentration of the two diisocyanates (TDI and MDI) (1000 mg per kg dry soil). Since both diisocyanates react with water, the absence of any effects may be due to the disappearance of the diisocyanates from the test medium.

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The two diamines (TDA and MDA) appeared to be more toxic than the corresponding diisocyanates. MDA was more toxic for the worm *Eisenia fetida* than TDA.

At a concentration of 464 mg TDA per kg of dry soil, four worms died, whereas in the controls, and at the lower test concentrations no mortality was observed. This mortality did not deviated significantly at the 5% level from that in its controls. However, considering that control mortality is only seldom observed in tests with the worm *Eisenia fetida* at TNO (no control mortality in any of these four tests), the death of those 4 worms at 464 mg TDA per kg dry soil was probably induced by the test substance, TDA.

For TDA, the start weight of the worms in the controls was significantly higher (Dunnett-test, $p=0.05$) than those exposed to 100, 215 and 464 mg per kg dry soil. At 464 and 1000 mg per kg dry soil, the percentage weight decrease deviated significantly from that observed in the controls. At 464 and 1000 mg per kg dry soil, this percentage also deviated significantly ($p=0.01$) from that at 46.4 and 100 mg per kg dry soil, whereas the initial wet weights at these concentrations did not differ significantly from each other. Therefore, the increased weight loss at 464 and 1000 mg TDA per kg dry soil cannot be attributed to the difference in initial weights of the worms, but must be attributed to the test substance, TDA.

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4. REFERENCES

- 1) OECD Guideline for testing of chemicals
no. 207 'Earthworm, acute toxicity tests'
Organization for Economic Co-operation and Development, Paris (1984)
- 2) Toxicity for earthworms. Artificial soil test.
Appendix III of DG XI/128/82 Rev. 4 (EEC Intercomparison exercises).
- 3) Good Laboratory Practice in the testing of chemicals
Organization for Economic Co-operation and Development, Paris (1982).
- 4) Kooijman, S.A.L.M. (1981).
Parametric analyses of mortality rates in bioassays.
Water Res. 15, 107-119.

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5. RETENTION OF RECORDS AND SAMPLES

All the data generated and all other information relevant to the quality and integrity of these studies have been filed under the study references IMW-91-0032-01 (TDI), IMW-91-0033-01 (TDA), IMW91-0034-01 (MDI) and IMW-91-0036-01 (MDA) in the archives of the TNO Institute of Environmental Sciences, Schoemakerstraat 97, 2628 VK Delft, The Netherlands. These records will be retained for a period of at least ten years after the cover date of this report.

Samples of the test substances have been deposited under the sample references IMW-91-0032-A (TDI), IMW-91-0033-A (TDA), IMW-91-0034-A (MDI) and IMW-91-0036-A (MDA) in the sample archives of the TNO Institute of Environmental Sciences at the same address; these samples will be stored for a period of at least ten years.

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6. DEVIATIONS FROM THE PROTOCOL

The weights of the worms at the start of the tests were between 300 and 600 mg instead of 600 ± 100 mg, as stated in the protocols. This is in accordance with the OECD Guideline 207 (ref. 1) and the Draft EC Guideline (ref. 2).

The pH and moisture content were not determined at the highest concentration of the test substances to prevent the risk of volatilization of the test compounds during the determination of moisture content and to prevent contamination of the pH electrode during the pH measurement. The OECD Guideline 207 (ref. 1) and the Draft EC Guideline (ref. 2) do not prescribe these measurements.

After 7 days the test containers with worms and soil were not emptied to assess the survival and condition of the worms. Only the general conditions of the worms as far as could be assessed from the outside of the test containers, were recorded. This is also in accordance with the Draft EC Guideline (ref. 2).

For the tests with TDI, MDI and MDA, controls were only used in which the soil was treated with the solvent acetone, in a similar manner to the soils with the test substance. This is in accordance with the OECD Guideline 207 (ref. 1) and the Draft EC Guideline (ref. 2).

The laboratory product MDA was given TNO code SIE. However, until February 28, 1992 this test substance was erroneously given TNO code SID. Since the test substance which was originally allocated this code (MDA, commercial product), was removed from the TNO test substance list and furthermore was only slightly different from SIE, no consequences can be expected from this mistake.

The test containers of the final tests with TDA were not labelled with the test code, SIBEFZ. This lack in the labelling did not lead to mistakes, since at that time no other tests with worms were carried out in the same room.

In the protocols, the test substances TDI and TDA were indicated as TDI 80/20 and TDA 80/20 respectively. The abbreviation MDA for the test substance 4,4'-diaminodiphenylmethane, laboratory product, was not used in the protocol.



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ANNEX A COMPOSITION AND PROPERTIES OF TDI, TDA, MDI AND MDA

Annex A1 Composition and properties of TDI

DIVISION OF TECHNOLOGY FOR SOCIETY TNO
DEPARTMENT OF BIOLOGY

Form no.: MTB/EG/003
 For: Characterization of the test substance
 Belonging to: Standard operating procedure MTB/PG/003

Test substance name or code to be used in report: Toluene Diisocyanate RO/20
TDI RO/20

Storage conditions:

Storage temperature: ☒ freezer ☒ refrigerator ☐ room temperature ☒ special (specify) _____ °C
 Photostability: ☒ protected from light Expiry date: 6 months from sample date = 20-1-90

* delete where applicable

Characterization:

Physical appearance: colourless to yellowish liquid at room temperatureBoiling point: 247 °C at 760 mm Hg Melting point: 12.5 °C Density: 1.21 g/cm³Batch no.: 808 Quantity submitted: 1 kgActive ingredient: Toluene Diisocyanate (80 % 2,4 isomer/20 % 2,6 isomer)Carrier, solvent or diluting agent: ./.Percentage content of active ingredient: >99.9Nature and quantity of impurities: Chlorine containing aromatic substances

Solvent	Solubility	Maximum storage time of solution
water	<u>TDI reacts with water</u>	
acetone	<u>Yes</u>	<u>solution should be freshly prepared</u>
methanol	<u>?</u>	
ethanol	<u>TDI reacts with alcohols</u>	
dimethylsulphoxide	<u>TDI reacts with DMSO</u>	

Information on toxicity (acute toxicity, oral-, dermal or inhalation toxicity, skin- and eye irritation, sensitization, carcinogenicity, mutagenicity, etc.):

DIN Safety Data Sheet
 Bayer 043412/01
 31 October 1990

Is the test substance explosive, inflammable, corrosive:

Other special handling instructions:

Form completed by:

Signature:

Date:

TNO study no.:
 2900601

MTB 41 0032 03
 TNO



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Annex A2 Composition and properties of TDA

DIVISION OF TECHNOLOGY FOR SOCIETY TNO
DEPARTMENT OF BIOLOGY

Form no. : MTB/EG/003
 For : Characterization of the test substance
 Belonging to : Standard operating procedure MTB/PG/003

Test substance name or code to be used in report: Toluene Diamine 80/20
 TDA 80/20

Storage conditions:

Storage temperature: ☒ freezer ☒ refrigerator ☐ room temperature ☒ special (specify) _____

Photostability: ☒ good ☐ protect from light

Expiry date: 6 months from sample date

* delete where applicable

Characterization:

Physical appearance: brown solid

Boiling point: ca 288 °C at 760 mm Hg Melting point: ca 100 °C Density: ca 1 g/cm³

Batch no.: 12 Quantity submitted: 1 kg

Active ingredient: Toluene Diamine

Carrier, solvent or diluting agent: -/-

Percentage content of active ingredient: >99 %

Nature and quantity of impurities: high boiling residues

Solvent	Solubility	Maximum storage time of solution
water	yes (100 g/l)	solution should be freshly
acetone	"	prepared each time
methanol	"	
ethanol	"	
dimethylsulphoxide	not tested	

Information on toxicity (acute toxicity, oral-, dermal or inhalation toxicity, skin- and eye irritation, sensitization, carcinogenicity, mutagenicity, etc.):

Is the test substance explosive, inflammable, corrosive:

Other special handling instructions:

TIN Safety Sheet
 Payer 011405/05
 3 December 1990

Form completed by:

Signature:

Date:

TNO study no.:
 2900601

MTB 411-01033-01



RA92188IMWip

Annex A3 Composition and properties of MDI

DIVISION OF TECHNOLOGY FOR SOCIETY TNO
DEPARTMENT OF BIOLOGY

Form no. MTB/EG/003
 For: Characterization of the test substance
 Belonging to: Standard operating procedure MTB/PG/003

Test substance name or code to be used in report: Diphenyl-methane-diisocyanate *01

Storage conditions:

Storage temperature: ☒ freezer ☒ refrigerator ☒ room temperature ☒ special (specify) _____Photostability: ☒ good ☒ protect from light *Expiry date: max. 6 months storage time at
20-25°C

* delete where applicable

Characterization:

Physical appearance: dark-brown liquidBoiling point: 250 °C at 760 mm Hg Melting point: 0 °C Density: 1,23 g/cm³

Batch no. _____ Quantity submitted: _____

Active ingredient: Diphenyl-methane-diisocyanate (isomers and homologous)

Carrier, solvent or diluting agent: _____

Percentage content of active ingredient: 40-50% 4,4'-/2-4% 2,4'-/40-60% 3-Ring-IsomersNature and quantity of impurities: Traces of phenylisocyanate and monochlorobenzene

*1: decomposition

*2: partial crystallisation

Solvent	Solubility	Maximum storage time of solution
water	Reaction with water yields urea and CO ₂	
acetone	yes	
methanol	Reaction with methanol yields urethane and CO ₂	
ethanol	" " ethanol	
dimethylsulphoxide		

water: Reaction with water yields urea and CO₂acetone: yesmethanol: Reaction with methanol yields urethane and CO₂ethanol: " " ethanol

dimethylsulphoxide: _____

Information on toxicity (acute toxicity, oral-, dermal or inhalation toxicity, skin- and eye irritation, sensitization, carcinogenicity, mutagenicity, etc.):

Is the test substance explosive, inflammable, corrosive:

Other special handling instructions:

Safety Data Sheet

Bayer 044192/04

29 October 1990

Form completed by

Signature:

Date:

TNO study no:

M T B [] [] [] [] [] []

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Annex A4 Composition and properties of MDA

DIVISION OF TECHNOLOGY FOR SOCIETY TNO
DEPARTMENT OF BIOLOGY

Form no MTB/EG/003
For Characterization of the test substance
Belonging to Standard operating procedure MTB/PG/003

Test substance name or code to be used in report: _____

4,4'-diaminodiphenylmethane, laboratory product

Storage conditions:

Storage temperature: ☒ freezer ☒ refrigerator ☐ room temperature ☒ special (specify) _____Photostability: ☒ good ☒ protect from light

Expiry date: _____

* delete where applicable

Characterization:

Physical appearance: colourless to light yellow, solid lumps

Boiling point: 238/ °C at 4 mm Hg Melting point: 91-92 °C Density: ca. 1.3 gm/cm³
at 100 °C

Batch no: 8 Quantity submitted: 1

Active ingredient: 4,4'-diaminodiphenylmethane > 99,5 %

Carrier, solvent or diluting agent: none

Percentage content of active ingredient: > 99,5 %

Nature and quantity of impurities: 2,4'-diaminodiphenylmethane (trace)
higher molecular weight oligomers (trace)

Solvent	Solubility	Maximum storage time of solution
water	practically insoluble	
acetone	soluble	
methanol	very soluble	
ethanol	soluble	
dimethylsulphoxide	(unknown)	

Information on toxicity (acute toxicity, oral-, dermal or inhalation toxicity, skin- and eye irritation, sensitization, carcinogenicity, mutagenicity, etc.)

DIN Safety Sheet
Bayer 328794/05
3 December 1990

Is the test substance explosive, inflammable, corrosive

Other special handling instructions

Form completed by

Signature

Date:

TNO study no
90060*

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ANNEX B INDIVIDUAL TEST DATA

Table B1 Number of living worms and their average weight (with standard deviation) after exposure to several concentrations of the test substances (mg per kg of dry artificial soil).
Footnotes to this table are given on page 31.

Table B1.1 Data on survival and weight of worms exposed to TDI

Time (days)	0				7	14				average percentage weight increase (s.d.)
concentration (mg.kg ⁻¹)	no. of living worms	condi- tion ^{a)}	average weight (g)	s.d.	condi- tion soil ^{b)}	no. of living	condi- tion ^{a)}	average weight (g)	s.d.	
0	10	1	0.38	0.05	8	10	1	0.34	0.03	-4.5 (4.8)
	10	1	0.38	0.06	8	10	1	0.37	0.06	
	10	1	0.35	0.03	8	10	1	0.34	0.03	
	10	1	0.39	0.06	8	10	1	0.39	0.06	
1000	10	1	0.38	0.05	8	10	2	0.38	0.05	-2.4 (1.7)
	10	1	0.36	0.03	8	10	2	0.34	0.04	
	10	1	0.39	0.09	8	10	2	0.38	0.06	
	10	1	0.38	0.05	8	10	2	0.37	0.06	

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Table B1.2 Data on survival and weight of worms exposed to TDA

Time (days)	0				7	14				average percentage weight increase (s.d.)
concentration (mg.kg ⁻¹)	no. of living worms	condi- tion ^{a)}	average weight (g)	s.d.	condi- tion soil ^{b)}	no. of living	condi- tion ^{a)}	average weight (g)	s.d.	
0	10	1	0.41	0.05	8	10	1	0.41	0.06	2.6 (2.0)
	10	1	0.40	0.06	8	10	1	0.42	0.06	
	10	1	0.43	0.05	8	10	1	0.45	0.05	
	10	1	0.40	0.05	8	10	1	0.42	0.04	
46.4	10	1	0.40	0.06	8	10	2	0.41	0.06	2.7 (1.2)
	10	1	0.40	0.08	8	10	2	0.42	0.10	
	10	1	0.37	0.04	8	10	2	0.38	0.05	
	10	1	0.41	0.06	8	10	2	0.42	0.06	
100	10	1	0.37	0.04	8	10	2	0.38	0.06	3.6 (3.5)
	10	1	0.37	0.05	8	10	2	0.40	0.05	
	10	1	0.37	0.04	8	10	2	0.37	0.04	
	10	1	0.40	0.06	8	10	2	0.42	0.05	
215	10	1	0.40	0.06	8	10	2	0.42	0.06	2.5 (1.3)
	10	1	0.36	0.04	8	10	2	0.37	0.05	
	10	1	0.39	0.06	8	10	2	0.39	0.09	
	10	1	0.37	0.03	8	10	2	0.38	0.04	
464	10	1	0.37	0.05	9	9	3	0.30	0.09	-11.4** (5.9)
	0	1	0.40	0.05	9	8	3	0.36	0.07	
	0	1	0.36	0.03	9	10	2	0.33	0.04	
	0	1	0.38	0.06	9	9	3	0.35	0.08	
1000	10	1	0.39	0.06	10	5	4	0.35	0.05	-13.3** (7.4)
	10	1	0.40	0.07	10	4	3	0.31	0.06	
	10	1	0.38	0.03	10	7	3	0.33	0.04	
	10	1	0.36	0.05	10	6	3	0.34	0.02	

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Table B1.3 Data on survival and weight of worms exposed to MDI

Time (days)	0				7	14				average percentage weight increase (s.d.)
concentration (mg.kg ⁻¹)	no. of living worms	condi- tion ^{a)}	average weight (g)	s.d.	condi- tion soil ^{b)}	no. of living	condi- tion ^{a)}	average weight (g)	s.d.	
0	10	1	0.51	0.07	8	10	1	0.48	0.06	-7.5 (1.9)
	10	1	0.49	0.06	8	10	1	0.47	0.05	
	10	1	0.48	0.05	8	10	1	0.44	0.05	
	10	1	0.53	0.06	8	10	1	0.48	0.08	
1000	10	1	0.54	0.08	8	10	2	0.48	0.09	-5.1 (6.0)
	10	1	0.44	0.07	8	10	2	0.41	0.06	
	10	1	0.50	0.06	8	10	2	0.45	0.07	
	10	1	0.47	0.07	8	10	2	0.48	0.08	

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Table B1.4 Data on survival and weight of worms exposed to MDA

Time (days)	0				7	14				average percentage weight increase (s.d.)
concentration (mg.kg ⁻¹)	no. of living worms	condition ^{a)}	average weight (g)	s.d.	condition soil ^{b)}	no. of living	condition ^{a)}	average weight (g)	s.d.	
0	10	1	0.36	0.04	8	10	1	0.39	0.05	6.1 (2.3)
	10	1	0.37	0.05	8	10	1	0.39	0.07	
	10	1	0.35	0.04	8	10	1	0.37	0.05	
	10	1	0.35	0.03	8	10	1	0.37	0.06	
18	10	1	0.36	0.04	8	10	2	0.34	0.05	-3.5 (1.5)
	10	1	0.35	0.04	8	10	2	0.34	0.04	
	10	1	0.35	0.04	8	10	2	0.33	0.05	
	10	1	0.39	0.04	8	10	2	0.37	0.05	
32	10	1	0.37	0.04	8	10	2	0.40	0.03	-1.9 (8.3)
	10	1	0.36	0.05	8	10	2	0.36	0.06	
	10	1	0.40	0.05	8	10	2	0.35	0.06	
	10	1	0.39	0.05	8	10	2	0.37	0.06	
56	10	1	0.36	0.04	8	10	2	0.34	0.02	-6.9* (0.8)
	10	1	0.37	0.06	8	10	2	0.34	0.03	
	10	1	0.37	0.04	8	10	2	0.34	0.03	
	10	1	0.36	0.05	8	10	2	0.34	0.05	
100	10	1	0.38	0.04	9	10	5	0.33	0.04	-13.7** (4.1)
	10	1	0.35	0.04	9	10	5	0.32	0.04	
	10	1	0.38	0.04	9	10	5	0.32	0.04	
	10	1	0.38	0.05	9	10	5	0.31	0.04	
180	10	1	0.37	0.03	11	10	6	0.29	0.02	-20.0** (1.6)
	10	1	0.34	0.04	11	9	6	0.27	0.04	
	10	1	0.34	0.03	11	10	6	0.28	0.03	
	10	1	0.36	0.04	11	10	6	0.29	0.05	
320	10	1	0.36	0.06	12	6	7	0.26	0.01	-19.6** (7.1)
	10	1	0.33	0.03	12	7	7	0.27	0.04	
	10	1	0.35	0.05	12	9	7	0.29	0.05	
	10	1	0.34	0.03	12	10	7	0.30	0.04	
560	10	1	0.36	0.03	12	5	7	0.21	0.07	-40.4** (13.0)
	10	1	0.35	0.03	12	4	7	0.26	0.02	
	10	1	0.36	0.06	12	0	-	--	--	
	10	1	0.36	0.04	12	2	7	0.17	0.06	

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- a) Explanation of the codes used in the description of the condition of the test animals.
1. appearance and behaviour of worms normal (visually estimated)
 2. appearance and behaviour of worms equal to those of the control worms
 3. appearance and behaviour of worms not equal to those of the control worms, they seemed to be somewhat smaller
 4. appearance and behaviour of worms not equal to those of the control worms, they were somewhat sluggish
 5. appearance and behaviour of worms not equal to those of the control worms, they seemed to be slightly more flabby than the control and the soil was slightly less well burrowed.
 6. appearance and behaviour of worms not equal to those of the control worms, they were sluggish and the soil was less well burrowed.
 7. appearance and behaviour of worms not equal to those of the control worms, they were very sluggish and wetter.
- b) Explanation of the codes used in the description of the condition of the soil in the test containers after 7 days.
8. soil appears to be normal, no death worms observed
 9. soil seemed to be slightly less burrowed
 10. soil was less burrowed, death animals were observed
 11. soil was less burrowed
 12. soil was less burrowed, indication of traces of death worms
- * weight increase/decrease is significantly (two-tailed Dunnett test, $p=0.95$) different from that of the control worms.
- ** weight increase/decrease is significantly (two-tailed Dunnett test, $p=0.99$) different from that of the control worms.

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Table B2 Individual weights of the worms (in g) at the start of the experiment ($t=0$) and at the end ($t=14$ days).

Table B2.1 Data on the weight of worms exposed to TDI

concentration (mg.kg ⁻¹)	vessel	weight (g) per worm at the start of the experiment ($t=0$).									
0	A	0.404	0.381	0.347	0.347	0.486	0.340	0.378	0.395	0.304	0.402
	B	0.334	0.425	0.366	0.437	0.399	0.322	0.392	0.486	0.326	0.317
	C	0.321	0.360	0.400	0.317	0.337	0.322	0.368	0.343	0.350	0.416
	D	0.367	0.334	0.419	0.481	0.444	0.346	0.425	0.448	0.306	0.348
1000	A	0.366	0.443	0.426	0.399	0.373	0.423	0.310	0.349	0.301	0.402
	B	0.345	0.312	0.353	0.370	0.419	0.365	0.352	0.356	0.378	0.327
	C	0.311	0.338	0.321	0.480	0.376	0.389	0.475	0.314	0.545	0.310
	D	0.466	0.377	0.299	0.340	0.351	0.354	0.361	0.365	0.454	0.425

concentration (mg.kg ⁻¹)	vessel	weight (g) per worm at the end of the experiment ($t=14$)									
0	A	0.326	0.300	0.313	0.360	0.375	0.313	0.364	0.337	0.364	0.295
	B	0.424	0.412	0.424	0.307	0.466	0.350	0.274	0.368	0.317	0.331
	C	0.348	0.356	0.347	0.351	0.311	0.326	0.303	0.397	0.335	0.352
	D	0.311	0.441	0.346	0.458	0.434	0.313	0.408	0.455	0.422	0.321
1000	A	0.450	0.361	0.366	0.366	0.346	0.396	0.272	0.413	0.393	0.405
	B	0.354	0.379	0.341	0.334	0.366	0.271	0.412	0.313	0.297	0.342
	C	0.501	0.375	0.428	0.407	0.338	0.314	0.322	0.338	0.386	0.368
	D	0.321	0.364	0.402	0.419	0.355	0.452	0.432	0.374	0.252	0.343

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Table B2.2 Data on the weight of worms exposed to TDA

concentration (mg.kg ⁻¹)	vessel	weight (g) per worm at the start of the experiment (t=0).									
0	A	0.408	0.425	0.338	0.371	0.422	0.420	0.498	0.421	0.465	0.350
	B	0.458	0.386	0.470	0.367	0.425	0.462	0.325	0.393	0.431	0.315
	C	0.510	0.435	0.358	0.424	0.482	0.395	0.452	0.418	0.382	0.449
	D	0.385	0.456	0.340	0.442	0.484	0.373	0.358	0.373	0.438	0.392
46.4	A	0.467	0.390	0.468	0.321	0.359	0.316	0.429	0.434	0.372	0.471
	B	0.356	0.354	0.423	0.457	0.342	0.518	0.342	0.556	0.339	0.355
	C	0.404	0.326	0.380	0.388	0.406	0.335	0.422	0.377	0.342	0.311
	D	0.476	0.389	0.360	0.430	0.435	0.512	0.331	0.387	0.349	0.381
100	A	0.305	0.382	0.306	0.331	0.387	0.405	0.387	0.429	0.385	0.394
	B	0.379	0.344	0.436	0.348	0.487	0.364	0.378	0.344	0.344	0.317
	C	0.370	0.381	0.407	0.374	0.363	0.318	0.369	0.309	0.372	0.423
	D	0.483	0.463	0.380	0.487	0.312	0.391	0.429	0.352	0.331	0.372
215	A	0.360	0.490	0.465	0.359	0.491	0.393	0.322	0.411	0.330	0.413
	B	0.357	0.374	0.417	0.343	0.415	0.292	0.372	0.323	0.373	0.317
	C	0.303	0.307	0.402	0.452	0.338	0.464	0.405	0.376	0.416	0.447
	D	0.366	0.366	0.339	0.331	0.398	0.334	0.396	0.406	0.383	0.352
464	A	0.345	0.389	0.359	0.334	0.411	0.316	0.472	0.389	0.398	0.327
	B	0.472	0.327	0.452	0.358	0.375	0.433	0.385	0.419	0.378	0.388
	C	0.327	0.364	0.364	0.397	0.380	0.323	0.354	0.350	0.312	0.402
	D	0.319	0.399	0.357	0.529	0.351	0.373	0.376	0.432	0.326	0.344
1000	A	0.358	0.377	0.505	0.344	0.342	0.475	0.340	0.409	0.398	0.366
	B	0.379	0.478	0.319	0.456	0.416	0.381	0.304	0.371	0.533	0.360
	C	0.375	0.370	0.434	0.346	0.323	0.356	0.384	0.390	0.403	0.415
	D	0.331	0.436	0.435	0.348	0.307	0.359	0.333	0.401	0.355	0.317

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concentration (mg.kg ⁻¹)	vessel	weight (g) per worm at the end of the experiment (t=14).									
0	A	0.465	0.345	0.418	0.408	0.508	0.340	0.395	0.408	0.347	0.469
	B	0.450	0.467	0.360	0.344	0.401	0.447	0.327	0.426	0.505	0.442
	C	0.500	0.386	0.491	0.428	0.416	0.383	0.546	0.451	0.416	0.470
	D	0.472	0.459	0.435	0.388	0.447	0.412	0.339	0.423	0.397	0.395
46.4	A	0.349	0.409	0.390	0.351	0.508	0.447	0.410	0.328	0.393	0.484
	B	0.367	0.545	0.326	0.566	0.509	0.334	0.301	0.400	0.471	0.351
	C	0.415	0.322	0.340	0.369	0.435	0.359	0.457	0.336	0.427	0.336
	D	0.349	0.361	0.399	0.382	0.405	0.558	0.423	0.427	0.494	0.408
100	A	0.381	0.469	0.304	0.404	0.440	0.380	0.312	0.382	0.300	0.405
	B	0.365	0.506	0.342	0.404	0.398	0.409	0.434	0.365	0.429	0.357
	C	0.338	0.385	0.312	0.317	0.427	0.401	0.367	0.362	0.410	0.351
	D	0.415	0.433	0.423	0.341	0.424	0.402	0.481	0.475	0.482	0.354
215	A	0.337	0.467	0.477	0.516	0.386	0.376	0.351	0.415	0.467	0.373
	B	0.289	0.328	0.436	0.419	0.394	0.347	0.360	0.390	0.407	0.322
	C	0.296	0.551	0.345	0.437	0.453	0.470	0.395	0.372	0.262	0.341
	D	0.463	0.367	0.386	0.370	0.355	0.329	0.389	0.383	0.364	0.375
464	A	0.398	0.279	0.391	0.397	0.327	0.267	0.309	0.203	0.120	
	B	0.435	0.382	0.356	0.411	0.412	0.356	0.306	0.214		
	C	0.388	0.342	0.369	0.275	0.319	0.314	0.308	0.275	0.298	0.330
	D	0.382	0.396	0.511	0.388	0.303	0.369	0.247	0.254	0.288	
1000	A	0.376	0.265	0.370	0.340	0.404					
	B	0.363	0.326	0.318	0.218						
	C	0.353	0.367	0.297	0.332	0.270	0.357	0.315			
	D	0.320	0.329	0.336	0.358	0.340	0.363				

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Table B2.3 Data on the weight of worms exposed to MDI

concentration (mg.kg ⁻¹)	vessel	weight (g) per worm at the start of the experiment (t=0).									
0	A	0.444	0.565	0.581	0.614	0.471	0.540	0.492	0.435	0.434	0.533
	B	0.521	0.416	0.532	0.602	0.409	0.429	0.501	0.479	0.515	0.523
	C	0.541	0.449	0.472	0.464	0.467	0.455	0.541	0.537	0.397	0.506
	D	0.557	0.511	0.556	0.522	0.619	0.435	0.550	0.520	0.559	0.420
1000	A	0.648	0.588	0.567	0.572	0.387	0.569	0.562	0.513	0.520	0.439
	B	0.438	0.558	0.326	0.527	0.399	0.467	0.403	0.482	0.416	0.357
	C	0.503	0.502	0.486	0.442	0.389	0.588	0.573	0.454	0.498	0.510
	D	0.385	0.535	0.498	0.429	0.526	0.472	0.571	0.467	0.345	0.432

concentration (mg.kg ⁻¹)	vessel	weight (g) per worm at the end of the experiment (t=14).									
0	A	0.531	0.395	0.485	0.569	0.450	0.475	0.478	0.415	0.541	0.456
	B	0.523	0.456	0.521	0.446	0.522	0.428	0.463	0.382	0.499	0.405
	C	0.412	0.397	0.392	0.423	0.411	0.437	0.557	0.422	0.504	0.402
	D	0.368	0.556	0.466	0.526	0.442	0.597	0.570	0.465	0.391	0.438
1000	A	0.514	0.609	0.549	0.563	0.433	0.471	0.423	0.399	0.536	0.328
	B	0.476	0.341	0.378	0.416	0.460	0.469	0.449	0.434	0.378	0.315
	C	0.485	0.428	0.537	0.569	0.417	0.384	0.359	0.377	0.507	0.481
	D	0.521	0.585	0.487	0.353	0.597	0.412	0.486	0.422	0.503	0.461

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Table B2.4 Data on the weight of worms exposed to MDA

concentration (mg.kg ⁻¹)	vessel	weight (g) per worm at the start of the experiment (t=0).									
0	A	0.327	0.338	0.414	0.350	0.408	0.351	0.302	0.363	0.378	0.318
	B	0.419	0.313	0.455	0.419	0.314	0.409	0.376	0.357	0.332	0.342
	C	0.304	0.390	0.335	0.327	0.432	0.337	0.347	0.315	0.356	0.348
	D	0.311	0.376	0.378	0.303	0.383	0.341	0.368	0.322	0.347	0.321
18	A	0.304	0.333	0.356	0.345	0.388	0.428	0.392	0.317	0.319	0.383
	B	0.366	0.370	0.301	0.358	0.363	0.324	0.343	0.311	0.304	0.415
	C	0.321	0.377	0.326	0.297	0.352	0.399	0.313	0.387	0.368	0.308
	D	0.443	0.423	0.364	0.427	0.357	0.333	0.399	0.369	0.389	0.345
32	A	0.465	0.334	0.351	0.351	0.363	0.380	0.378	0.322	0.385	0.387
	B	0.387	0.441	0.302	0.341	0.318	0.409	0.436	0.350	0.336	0.305
	C	0.421	0.336	0.501	0.390	0.441	0.306	0.399	0.397	0.374	0.408
	D	0.421	0.321	0.401	0.458	0.338	0.386	0.424	0.349	0.336	0.409
56	A	0.349	0.349	0.324	0.347	0.465	0.321	0.344	0.378	0.324	0.371
	B	0.301	0.316	0.361	0.436	0.355	0.355	0.483	0.378	0.344	0.328
	C	0.389	0.413	0.301	0.336	0.316	0.366	0.404	0.419	0.381	0.355
	D	0.305	0.380	0.329	0.358	0.422	0.302	0.311	0.364	0.393	0.423
100	A	0.436	0.354	0.306	0.407	0.392	0.433	0.344	0.413	0.376	0.332
	B	0.377	0.430	0.348	0.300	0.365	0.300	0.362	0.351	0.325	0.370
	C	0.388	0.383	0.342	0.387	0.359	0.340	0.456	0.368	0.428	0.304
	D	0.323	0.460	0.356	0.358	0.387	0.322	0.429	0.465	0.530	0.367
180	A	0.339	0.387	0.337	0.436	0.342	0.379	0.353	0.352	0.367	0.393
	B	0.333	0.339	0.417	0.302	0.312	0.379	0.359	0.317	0.366	0.316
	C	0.376	0.301	0.338	0.316	0.306	0.358	0.344	0.390	0.324	0.340
	D	0.310	0.317	0.325	0.363	0.441	0.374	0.368	0.384	0.359	0.370
320	A	0.434	0.328	0.314	0.335	0.326	0.309	0.307	0.406	0.462	0.380
	B	0.302	0.338	0.335	0.338	0.318	0.318	0.325	0.303	0.400	0.367
	C	0.434	0.394	0.359	0.452	0.324	0.321	0.320	0.320	0.311	0.307
	D	0.355	0.359	0.300	0.381	0.312	0.322	0.343	0.313	0.346	0.326
560	A	0.371	0.372	0.321	0.344	0.337	0.392	0.410	0.349	0.340	0.364
	B	0.355	0.313	0.366	0.373	0.377	0.356	0.341	0.325	0.386	0.324
	C	0.479	0.390	0.325	0.388	0.303	0.327	0.336	0.306	0.441	0.326
	D	0.372	0.314	0.327	0.375	0.345	0.369	0.405	0.408	0.310	0.339

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concentration (mg/kg)	vessel	weight (g) per worm at the end of the experiment (t=14).									
0	A	0.313	0.364	0.389	0.432	0.489	0.363	0.417	0.342	0.372	0.397
	B	0.508	0.371	0.421	0.322	0.477	0.441	0.299	0.309	0.345	0.389
	C	0.346	0.345	0.362	0.353	0.331	0.318	0.385	0.479	0.402	0.337
	D	0.434	0.335	0.399	0.391	0.286	0.350	0.348	0.300	0.363	0.465
18	A	0.361	0.305	0.344	0.304	0.371	0.328	0.319	0.341	0.448	0.295
	B	0.378	0.343	0.351	0.382	0.271	0.347	0.375	0.315	0.320	0.353
	C	0.347	0.244	0.322	0.294	0.405	0.415	0.305	0.341	0.303	0.332
	D	0.439	0.410	0.391	0.405	0.381	0.299	0.372	0.335	0.342	0.304
32	A	0.396	0.465	0.399	0.419	0.408	0.358	0.402	0.386	0.383	0.426
	B	0.395	0.290	0.351	0.389	0.335	0.384	0.275	0.338	0.337	0.491
	C	0.390	0.347	0.334	0.371	0.463	0.295	0.357	0.400	0.294	0.270
	D	0.292	0.488	0.407	0.417	0.374	0.359	0.348	0.314	0.314	0.382
56	A	0.352	0.292	0.350	0.317	0.319	0.372	0.352	0.334	0.333	0.324
	B	0.367	0.324	0.299	0.359	0.349	0.349	0.393	0.316	0.321	0.297
	C	0.380	0.355	0.343	0.274	0.350	0.370	0.355	0.360	0.297	0.325
	D	0.410	0.354	0.333	0.377	0.294	0.351	0.264	0.365	0.260	0.360
100	A	0.309	0.300	0.305	0.279	0.313	0.344	0.313	0.333	0.390	0.388
	B	0.334	0.371	0.273	0.368	0.296	0.305	0.294	0.396	0.279	0.316
	C	0.290	0.288	0.369	0.299	0.307	0.362	0.277	0.353	0.386	0.292
	D	0.378	0.296	0.362	0.264	0.259	0.292	0.286	0.347	0.295	0.318
180	A	0.321	0.288	0.335	0.284	0.270	0.315	0.294	0.294	0.270	0.264
	B	0.252	0.281	0.257	0.304	0.263	0.228	0.349	0.216	0.276	
	C	0.305	0.258	0.273	0.275	0.251	0.285	0.248	0.314	0.313	0.265
	D	0.257	0.314	0.273	0.259	0.260	0.325	0.227	0.334	0.370	0.259
320	A	0.241	0.265	0.259	0.255	0.252	0.268				
	B	0.325	0.301	0.281	0.220	0.245	0.272	0.244			
	C	0.230	0.271	0.296	0.296	0.344	0.237	0.264	0.282	0.366	
	D	0.373	0.256	0.265	0.273	0.313	0.346	0.286	0.246	0.284	0.333
560	A	0.237	0.143	0.141	0.287	0.259					
	B	0.236	0.260	0.279	0.249						
	C										
	D	0.121	0.212								

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ANNEX C MATERIALS USED FOR THE PREPARATION OF THE ARTIFICIAL SOIL

Sphagnum peat

Pindstrup Sphagnum originating from Denmark

Fa. Jongkind B.V.

Oosteinderweg 357, Aalsmeer, The Netherlands

Arrival date at TNO: December 7, 1990

Fine industrial sand

M32

Fa. Van Loon

Wierselaan 121, Vreeswijk, The Netherlands

Arrival date at TNO: January 1, 1992

Kaolin clay

China Clay Ast. containing 85-90% Kaolin

Fa. Vingerling B.V.

Provincialeweg West 44, Haastrecht, The Netherlands

Arrival date at TNO: February 13, 1990



ANNEX D ESTIMATION OF THE LC50 AND ITS CONFIDENCE INTERVAL

At a given time, the mortality probability of an individual is assumed to be logistically related to the logarithm of the concentration, i.e.

$$p_i = \frac{e_i + p_0}{1 + e_i}, \text{ where } e_i = (c_i/\alpha)^{1/\beta} \text{ and}$$

p_i is the mortality probability in the i^{th} concentration

p_0 is the mortality probability in concentration 0

α is the LC50

β is a parameter inversely proportional to the maximum gradient of the dose response function

c_i is the i^{th} concentration.

The parameters p_0 , α and β are estimated from the counts by means of the maximum likelihood method; i.e. the parameter values to be selected maximize the probability of the counts as a function of the three parameters. Since the distribution of α will not be symmetrical, the variance-covariance matrix is not estimated for the parameters p_0 , α and β themselves, but for p_0 , $\gamma = \ln \alpha$ and β . The variance-covariance matrix is estimated by the inverse of the information matrix.

The 95% confidence limits of the LC50 are now given by

$$\alpha \cdot \exp(\pm 2 [\text{var}(\gamma)]^{1/2}) = \alpha \cdot \exp(\pm 2 [\text{var}(\ln \alpha)]^{1/2}).$$

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(Month) (Day) (Year) Camera Operator

Place Syracuse New York
(City) (State)

